Temporal changes in immune blood cell parameters in COVID-19 infection and recovery from severe infection

Since emerging in China in December 2019, the infection caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), coronavirus disease 2019 (COVID-19), has infected >3 million people worldwide (Johns Hopkins University and Medicine: Coronavirus Resource Center). Since then, clusters of infections have emerged in Singapore, particularly among younger workers in communal living environments. The spectrum of COVID-19 infection ranges from a mild respiratory illness, to viral pneumonia and life-threatening acute respiratory distress syndrome in up 15–20%.[1,2] Advanced age, comorbidities and an immunocompromised state are consistent risk factors for poor outcomes.[1] Biomarkers associated with severe disease and mortality includes: lymphopenia, thrombocytopenia, higher leucocyte counts, elevated C-reactive protein, D-dimer, and a host of inflammatory cytokines.[1]

We hypothesised that additional lymphocyte parameters, which can be obtained using haematology analysers, may provide useful information on the status of the immune system during infection and might correlate with disease outcome. The Sysmex XN analysers (Sysmex Corp., Kobe, Japan) utilise fluorescence flow cytometry for the leucocyte differential, allowing for enhanced subset differentiation based on forward scatter, side scatter and RNA content.[3] Activated lymphocytes have fluorescence intensity above that of normal lymphocytes and a subset of these with the highest fluorescence have been shown to be antibody-synthesising lymphoplasmacytoid B cells and plasma cells.[4]

We conducted an observational cohort study of patients with COVID-19 in three tertiary hospitals of the National University Health System (Ethics approval Domain Specific Review Board (DSRB) 2020/00310) to determine disease associations. Consecutive patients with COVID-19 infection confirmed by positive SARS-CoV-2 polymerase chain reaction (PCR) on respiratory samples and having complete outcome data were studied. Patients were confined until two consecutive PCR-negative nasal swabs and clinical recovery before being discharged. Case severity was classified as mild, severe and critical according to the criteria of Wu and McGoogan.[2]

Full blood count (FBC) was assessed on the day of admission, with unwell patients having serial FBCs based on clinical indications. The FBC sample was analysed on Sysmex XN-10 analyser (Sysmex Corp.), which identified reactive lymphocytes (RE-Lymph) and antibody-synthesising lymphocytes (AS-Lymph). Reference ranges were derived from 120 healthy donors. Statistical analysis was performed by using Stata Statistical Software, release 16.1 (StataCorp., College Station, TX, USA) and the level of significance was set at 5%. Two-sided unpaired *t*-tests and Mann–Whitney *U*-tests were used for parametric and non-parametric data. Pearson's correlation or Spearman rank correlation was used to assess the associations between variables depending on their normality.

A total of 76 cases, 56 mild, 20 severe/critical (SC), were studied. Three mild cases with no FBC were excluded from the analysis. As shown in Table I, patients in the SC group were older and had a higher incidence of comorbidities. The median day of intubation for SC patients was day 10 of illness and median duration was 11 days. The platelet count was lower and the neutrophil count higher in the SC group than in the mild disease group. The lymphocyte count was significantly lower in the SC group, but RE-Lymph and AS-Lymph subsets were significantly higher in the SC group.

The changes in FBC parameters during infection among SC cases are shown in Fig 1 as median values from illness onset. White blood cell (WBC), neutrophil and platelet counts trended downward to a nadir at day 8–9 of illness but gradually recovered in the subsequent days (Fig 1A). The trends of the lymphocyte and lymphocyte subsets (Fig 1B,C) showed that while the lymphocyte count decreased gradually, the proportion of RE-Lymph and AS-Lymph progressively increased towards day 15–16. In mild cases, significant correlations were found between lymphocyte parameters and the day of illness on which the initial FBC was performed. Lymphocyte count ($r = 0{\cdot}3712$, $P = 0{\cdot}0062$), RE-Lymph count ($r = 0{\cdot}495$, $P = 0{\cdot}002$), RE-Lymph% (0·4639, $P = 0{\cdot}005$) and RE-Lymph as a percentage of lymphocytes (RE-Lymph%/L) ($r = 0{\cdot}3228$, $P = 0{\cdot}0196$) increased with number of days of illness, while no significant correlation was found for WBC, neutrophil, platelet and AS-Lymph parameters.

Among all FBC parameters, AS-Lymph as a percentage of lymphocytes (AS-Lymph%/L) yielded the best area under the receiver operating characteristic (ROC) curve (0·71) for predicting severe disease. A value >1·6% gave a sensitivity and specificity of 75% and 67%. A table of the ROC analysis for lymphocyte parameters is presented in the supplementary section (Table S1).

The immunopathology of severe COVID-19 is the result of an excessive dysregulated immune response,[5] while

doi: 10.1111/bjh.16847

Table I. Clinical characteristics and FBC parameters at presentation.

Characteristic	All patients (n = 76)	Severe/critical (n = 20)	Mild (n = 56)	P*
Age, years, median (range)	46 (19–71)	58 (36–70)	38 (19–71)	0·0002
Women, n (%)	32 (42·1)	6 (30·0)	26 (46·4)	
Ethnicity, n (%)				
Chinese	52 (68·4)	17 (85)	35 (62·5)	
Malay	4 (5·3)	2 (10)	2 (3·6)	
Indian	3 (3·9)	1 (5)	2 (3·6)	
Caucasian	11 (14·5)	0 (0)	11 (19·6)	
Others	6 (7·9)	0 (0)	6 (10·7)	
Major illness, n (%)				
Hypertension	19 (25·0)	8 (40)	11 (19·6)	0·070
Ischaemic heart disase	19 (25·0)	8 (40)	11 (19·6)	0·070
Dyslipidaemia	2 (2·6)	1 (5)	1 (1·8)	0·440
Heart failure	1 (1·7)	1 (5)	0 (0)	
Previous strokes	1 (1·7)	1 (5)	0 (0)	
Diabetes	8 (10·5)	5 (25)	3 (5·4)	0·014
Renal impairment	3 (3·9)	2 (10)	1 (1·8)	0·110
Day of illness onset at presentation, days, median (range)	5 (1–15)	6 (1–14)	4 (1–15)	0·444
Median (range)	Reference range			
Creatinine, μmol/l	60–107 (Male) 50–90 (Female)	70 (41–502)	89 (70–502)	71 (41–134)	
LDH, u/l	250–580	401 (135–6374)	403 (290–6374)	398 (135–849)	
WBC, × 10^9/l	6·92 (5·13–9·86)	5·27 (2·60–18·55)	6·11 (3·48–16·39)	5·16 (2·6–18·55)	0·1533
Haemoglobin, g/l	13·1–16·6 (Male) 11·4–14·7 (Female)	14·6 (8·7–16·8)	15·05 (8·7–15·5)	14·4 (0·98–16·8)	0·1758
PLT#, × 10^9/l	261 (201–364)	205 (64–400)	173·5 (64–299)	221 (140–400)	0·0003
NEUT#, × 10^9/l	3·90 (2·64–5·97)	3·39 (1·10–15·05)	4·56 (1·79–14·75)	3·15 (0·98–16·76)	0·0054
LYMPH#, × 10^9/l	2·22 (1·59–3·50)	1·30 (0·43–3·88)	1·015 (0·43–2·36)	1·34 (0·54–3·88)	0·0089
MONO#, × 10^9/l	0·55 (0·28–1·02)	0·52 (0·17–1·36)	0·48 (0·17–1·36)	0·54 (0·19–1·35)	0·3744
EO#, × 10^9/l	0·19 (0·05–1·10)	0·04 (0–0·42)	0·01 (0–0·24)	0·05 (0–0·42)	0·0019
BASO#, × 10^9/l	0·04 (0·01–0·11)	0·02 (0–0·1)	0·01 (0–0·10)	0·02 (0–0·09)	0·0056
AS-LYMPH#, × 10^9/l	0·01 (0–0·03)	0·01 (0–0·15)	0·02 (0–0·15)	0·01 (0–0·12)	0·0329
AS-LYMPH%, %	0·1 (0–0·39)	0·30 (0–2·3)	0·4 (0–1·6)	0·2 (0–2·3)	0·0949
RE-LYMPH#, × 10^9/l	0·05 (0·01–0·18)	0·04 (0–0·27)	0·04 (0·01–0·27)	0·04 (0–0·21)	0·5195
RE-LYMPH%, %	0·6 (0·2–2·4)	0·8 (0·01–3·3)	0·75 (0·1–2·30)	0·8 (0–3·3)	0·9427
-AS-LYMPH%/L, %	0·41 (0–1·11)	0·9 (0–24·6)	2·15 (0–24·6)	0·6 (0–8·1)	0·0014
-RE-LYMPH%/L, %	1·92 (0·54–6·77)	3·35 (0·41–44·3)	5 (0·8–44·3)	2·85 (0–11·1)	0·0352

#, absolute count; %, percentage of white cells; %/L, percentage of lymphocytes; AS-LYMPH, antibody-synthesising lymphocytes; BASO, basophils; EO, eosinophil; LDH, lactate dehydrogenase; LYMPH, lymphocytes; NEUT, neutrophil; PLT, platelets; RE-LYMPH, reactive lymphocytes; WBC, white blood cell.

* Comparison between severe/critical cases and mild cases.

humoral immunity is thought to be essential in controlling the persistent phase of infection.[6] Wang *et al.*[7] highlighted an association between lymphopenia and severe disease, and our results showed likewise. Additionally, we found that the proportion of activated lymphocytes was clearly higher in severe disease. This increase in AS-Lymph correlates with lymphoplasmacytoid lymphocytes,[8] and $CD38^+$ antigen-secreting B cells in patients with COVID-19.[9] AS-Lymph increased particularly in the second week of illness, which parallels seroconversion in the second week as described by Zhao *et al.*[10]

Antibody enhancement may play a role in immunopathology, as higher titres were found in critical cases,[10] and we similarly observed higher AS-Lymph in SC cases. In contrast, Wan *et al.*[11] noted no difference in total $CD19^+$ B cells. Paradoxically, we also observed that the rise in AS-Lymph predated clinical recovery in several patients, suggesting that functional studies are required to determine if these cells are protective or immunopathogenic in the context of COVID-19. Due to the retrospective nature of our present study, we were unable to serially track FBC changes in mild cases. Our sample size is also not powered to explore independent

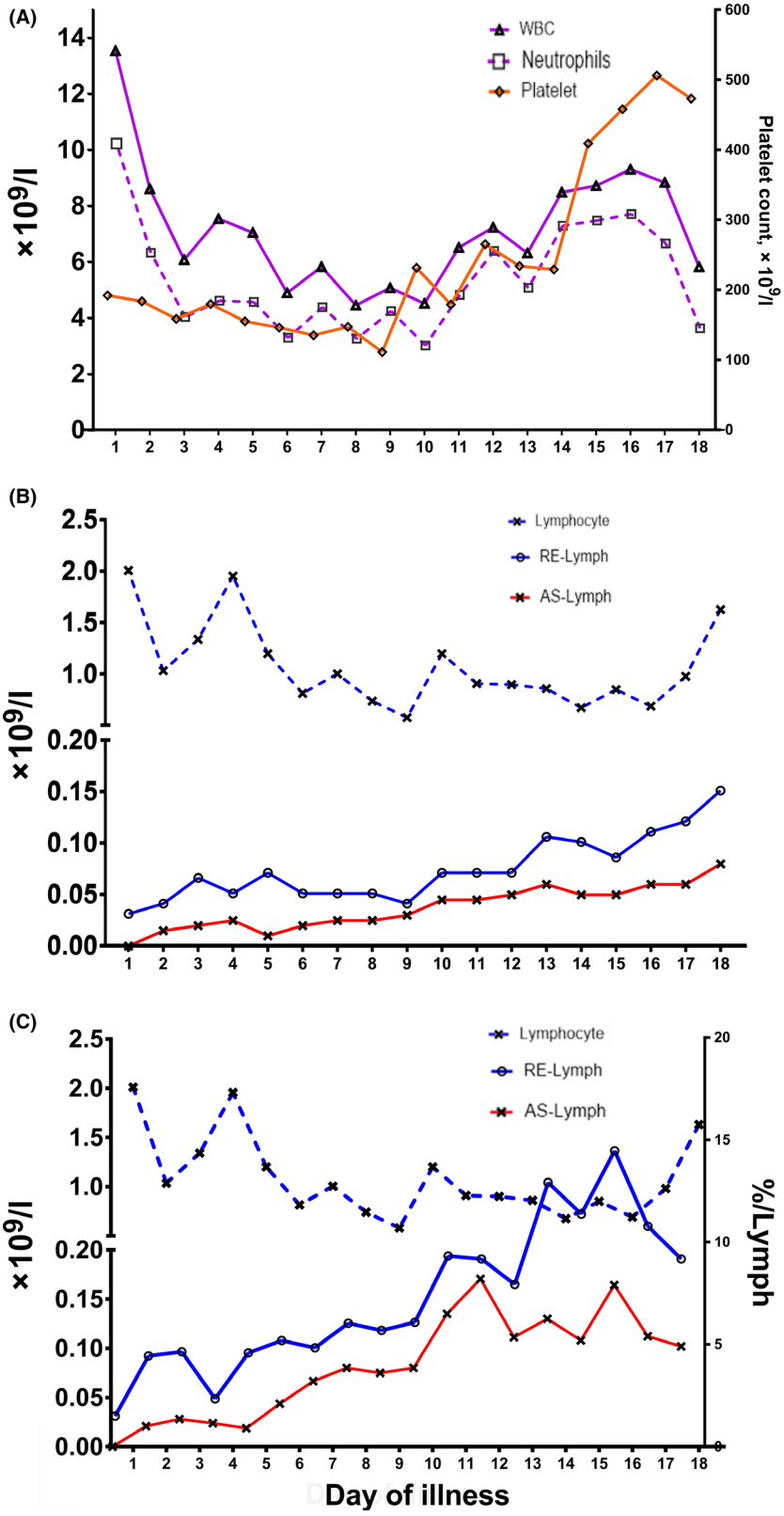

Fig 1. Time trend of haematological parameters in patients with confirmed COVID-19 with severe and critical illness. (A) White blood cell and neutrophil count (left axis), platelet count (right axis) decreased to its nadir level at day 8–9, but increased to normal ranges thereafter. (B) Absolute count of lymphocytes, reactive lymphocytes (RE-Lymph) and its subset antibody-synthesising lymphocytes (AS-Lymph) increased progressively up to day 16. (C) Relative percentage of RE-Lymph and AS-Lymph (right axis) were trended with the absolute lymphocyte count across the day of illness.

prognostic biomarkers. However, taken together, our present data show that the FBC and its extended parameters may be a valuable tool to triage patients with COVID-19 and provides evidence to further explore the role of lymphocyte subsets in this disease.

Acknowledgements

We thank the departments of Laboratory Medicine of National University Hospital, Ng Teng Fong General Hospital and Alexandra Hospital for supporting the laboratory data collection. We also greatly appreciate the efforts of healthcare workers and the support of their families during this outbreak.

Conflict of interest

All authors declare no competing interests.

Author contributions

Christina Y.C. Yip, Shir Ying Lee, Eng Soo Yap designed the study, acquired and analysed the data and wrote the paper. Winnie Z.Y. Teo, Chun-Tsu Lee and Sanjay De Mel, designed the study, acquired the data and contributed to the manuscript. Sheryl Kan, Melvin C.C. Lee and Will N.H. Loh acquired the data and critically reviewed the manuscript. Er Luen Lim analysed the data and critically reviewed the manuscript.

Christina Y. C. Yip[1]
Eng Soo Yap[1,2,3]
Sanjay De Mel[2]
Winnie Z. Y. Teo[2,4]
Chun-Tsu Lee[2,4]
Sheryl Kan[3]
Melvin C. C. Lee[5]
Will N. H. Loh[5]
Er Luen Lim[6]
Shir Ying Lee[1,2]

[1]Division of Haematology, Department of Laboratory Medicine, National University Hospital, [2]Department of Haematology-Oncology, National University Cancer Institute, [3]Division of Medicine, Ng Teng Fong General Hospital,Jurong Health, Singapore City, [4]Fast and Chronic Program, Alexandra Hospital, National University Health System, [5]Department of Anaesthesia, National University Hospital, and [6]Department of Emergency Medicine, National University Hospital, Singapore City, Singapore.
E-mail: shir_ying_lee@nuhs.edu.sg

Keywords: COVID-19, antibody-synthesising lymphocytes, full blood count, disease, severity, lymphocytes

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. ROC analysis for lymphocyte parameters in differentiating mild from severe and critical COVID-19.

References

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. 2020;**395**:497–506.
2. Wu Z, McGoogan JM. Characteristics of and important lessons from the coronavirus disease 2019 (COVID-19) outbreak in China: summary of a report of 72 314 cases from the Chinese Center for Disease Control and Prevention. *JAMA*. 2020;**323**:1239–42.
3. Sysmex Europe GmbH. Novel haematological parameters for rapidly monitoring the immune system response. Sysmex White Paper Infection/Inflammation. In: *Sysmex white paper Infection/inflammation*. 2017, pp. 1–5.
4. Linssen J, Jennissen V, Hildmann J, Reisinger E, Schindler J, Malchau G, et al. Identification and quantification of high fluorescence-stained lymphocytes as antibody synthesizing/secreting cells using the automated routine hematology analyzer XE-2100. *Cytometry B Clin Cytom*. 2007;**72**:157–66.
5. Shi Y, Wang Y, Shao C, Huang J, Gan J, Huang X, et al. COVID-19 infection: the perspectives on immune responses. *Cell Death Differ*. 2020;**27**:1451–4.
6. Li G, Fan Y, Lai Y, Han T, Li Z, Zhou P, et al. Coronavirus infections and immune responses. *J Med Virol*. 2020;**92**:424–32.
7. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, et al. Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus–infected Pneumonia in Wuhan, China. *JAMA*. 2020;**323**:1061–9.
8. Fan BE, Chong VC, Chan SS, Lim GH, Lim KG, Tan GB, et al. Hematologic parameters in patients with COVID-19 infection. *Am J Hematol*. 2020;**95**:E131–4.
9. Thevarajan I, Nguyen TH, Koutsakos M, Druce J, Caly L, van de Sandt CE, et al. Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19. *Nat Med*. 2020;**26**:453–5.
10. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y, et al. Antibody responses to SARS-CoV-2 in patients of novel coronavirus disease 2019. *Clin Infect Dis*. 2020 [Epub ahead of print]. DOI: https://doi.org/10.1093/cid/ciaa344.
11. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, et al. Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. *Br J Haematol*. 2020;**189**:428–37.